California Environmental Protection Agency

Air Resources Board

PROCEDURE FOR THE ANALYSIS OF PARTICULATE ANIONS AND CATIONS IN MOTOR VEHICLE EXHAUST BY ION CHROMATOGRAPHY

Standard Operating Procedure No. MLD 142
Revision 1.2

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1 Introduction

In 1998, the Air Resources Board (ARB) identified diesel particulate matter (PM) as a Toxic Air Contaminant. A Diesel Risk Reduction Plan was subsequently adopted to reduce PM emitted from diesel engines. The chemical characterization of diesel PM is critical to the understanding of the contribution of mobile sources to air pollution and health effects. The ARB has initiated motor vehicle testing programs to determine speciated PM emissions from in-use vehicles as well as those equipped with prototype emission control technologies. This test method will be used to determine anions and cations in the PM fraction of exhaust emissions.

2 Method Summary

SOP No. MLD 142 determines selected particulate anions (nitrate and sulfate) and cations (sodium, ammonium, and potassium) collected on filters from motor vehicle exhaust. The filters are extracted in deionized water by sonicating and storing extracts overnight in a refrigerator to settle particles. The extract is analyzed by ion chromatography using a system comprised of a guard column, an analytical column, a self-regenerating suppressor, and a conductivity detector. The peak integrations are conducted by a software program based on conductivity. Then, component concentrations are calculated based on peak areas using external standards. The test method is in large part identical to MLD 064, which is utilized for the determination of anions and cations in ambient PM samples.

3 Interferences and Limitations

3.1 Co-elution interference can be caused by ions with retention times that are similar to and thus overlap those of the ions of interest, or by large amounts of any one anion or cation that interferes with the peak resolution of an ion with a similar retention time. Coelution interferences may be reduced by diluting the sample.

3.2 Interferences may be caused by contaminants in the reagent water, reagents, glassware, filters, and other sample processing apparatus that could lead to an elevated baseline or to detectable concentrations of any of the ions of interest. Deionized (DI) water blank, extraction water blank, and a filter blank are run with each set of samples to monitor these possible sources of contamination.

3.3 Rough sample handling can lead to particulate loss. Most PM2.5 samples adhere well to the filter surface so particulate loss is not an obvious problem. If this method is used for PM10 particulate samples, more careful handling should be taken because some PM10 samples may have loose particles. However, analytical comments attached to the result

should be included if particle loss during handling is observed.

3.4 Incomplete ion extraction from the particulate matter can also lead to inaccurate results. Teflon filters are hydrophobic. Addition of small amounts of ethanol or other wetting agents can help the extraction. The use of sonic treatment and extensive mixing is used to enhance contact between particulate matter on filters and the extraction solution. In addition, the composition of the extraction solution affects extraction efficiencies. In our extraction evaluation experiments, the selected anions and cations on filters used for exhaust PM collection can be completely extracted with only DI water.

3.5 Changes in retention time and loss of resolution can be signs of column deterioration. Monitoring retention times and column back pressure will assist in determining when a column or guard column needs to be replaced.

4 Instrument and Apparatus

The Ion Chromatographic system Dionex IC LC20 is used for the analysis of anions and the Ion Chromatographic system Dionex ICS-2000 is used for the analysis of the cations. Therefore, most of instrument components and conditions, reagents, and materials are based on the Dionex system in this SOP. Alternative IC instruments of similar capabilities are acceptable. If alternative IC instruments are used, different parameters and reagents, etc. may be used.

This SOP assumes familiarity with the installation and operation of the IC instrument. For detailed instructions in the operation of the Dionex IC, refer to Dionex operations manuals.

4.1 The Dionex IC for the analysis of ions is comprised of the following modular units purchased from the Dionex Corporation:

- 4.1.1. LC20 Ion Chromatography System
  - 4.1.1.1. Gradient pump GP50
  - 4.1.1.2. Chromatography enclosure
  - 4.1.1.3. Conductivity detector CD25
- 4.1.2. ICS-2000 Ion Chromatography System
- 4.1.3. Automated sampler AS40 with 5 ml vial cassettes.

4.2 IC Operating conditions:

	ANIONS	CATIONS
Sample loop volume	100 µL/mL	25 µL/mL
Analytical columns	Dionex, IonPac AS14A	Dionex, IonPac CS12A-5µm
Guard columns	Dionex, IonPac AG14A	Dionex, IonPac CG12A-5µm
Self-Regenerating Suppresor	ASRS ULTRA II 4mm	CSRS Ultra II 2 mm
Eluent solutions	8.0 mM carbonate / 1.0 mM bicarbonate	20mM Methanesulfonic acid
Eluent flow rates	1.0 mL/min	0.5 mL/min

4.3 Other Equipment:

4.3.1. Acquisition software: Chromeleon, version 6.80
4.3.2. Bottle-top dispenser, 25.0 mL volume or 12 mL volume – calibrate by weight with glass stopped cylinder or volumetric flask.
4.3.3. Analytical balance
4.3.4. Pipetters with disposable pipette tips: 10-300 µL 100– 1000 µL and 100 – 5000 µL
4.3.5. Ultrasonic water bath

5 Reagents and Materials

5.1 Materials:

5.1.1 Volumetric flasks: 100, and 2000 mL sizes. Use glass to prepare the anion standards and PMP (polymethylpentene) Nalgene flask for the cation standards.
5.1.2 Disposable plastic centrifuge tubes with caps, 50 mL or 15 mL
5.1.3 Dionex 5 mL autosampler vials with 20 mm filter caps – Rinse vials and caps with DI water twice, soak in DI water and sonicate for 1 hour, rinse and soak in DI water for 24 hours, rinse with DI water and air dry in a covered plastic container. Clean vials and caps a few days before use.
5.1.4 Beaker, 150 mL size.
5.1.5 Disposable plastic pipets
5.1.6 High-purity helium (99.9995%) or High-purity nitrogen (99.9995%).
5.1.7 Latex Gloves, disposable, class 100 - Rinse with DI water and air dry before use.

5.2 Chemicals: All chemicals are at least spectrophotometric grade.

5.2.1. AS14A Eluent Concentrate (Part # 56937) (800.0 mM carbonate/100.0 mM bicarbonate.) from Dionex Corporation

5.2.2. Methanesulfonic acid (MSA) in EluGen Cartridge (Part # 58902) from Dionex Corporation

5.2.3. Nanopure ASTM Type 1 deionized water (resistance not less than 18 megohm per cm)

5.3 IC Eluents:

5.3.1 Stock eluent for the anions from Dionex, AS14A Eluent Concentrate (Part # 56937) (800.0 mM carbonate/100.0 mM bicarbonate.) To make 2 liters of eluent, pipet 20.0 mL of this concentrated eluent into a 2 L volumetric flask and dilute to a final volume of 2 L using nanopure deionized water. Mix well, sonicate the solution in the flask for 20 min to degas, and transfer it to the eluent reservoir (A) on top of the IC rack.

5.3.2 Methanesulfonic acid (MSA) in EluGen Cartridge (Part # 58902) from Dionex Corporation for the cation eluent generator. The eluent generator mixes the MSA with nanopure deionized water to produce a concentration of 20mM MSA.

5.4 Standards:

5.4.1 NIST Traceable Stock Standards:

Anion and cation stock solution concentrations are National Institute of Science and Technology (NIST) traceable. Four stock solutions are purchased from Inorganic Ventures, two for making working standards and the other two for making working controls. To avoid systematic errors, the four stock solutions must have different lot numbers. The concentration of all stock standards is 1000 μg/mL for each ion. All standard and control solutions are stored in the refrigerator until ready for use.

5.4.2 Anion and cation working standards:

The following table lists the dilutions used to prepare the working standards for both the anion and cation analyses. 100 mL volumetric flasks are used for standard preparation. Stock standard solutions are transferred by pipetters. All dilutions are made using nanopure deionized water. Store the working standards in 50 mL disposable centrifuge tubes in the refrigerator. Working standards are usable for no more than 21 days before they must be prepared again from the stock solution.

Stock Concentration (µg/mL)	Volume of Stock Solution Needed (mL)	Final Dilution Volume (mL)	Final Concentration (µg/mL)	Working Standard No.
10	0.200	100	0.02	1
10	0.500	100	0.05	2
10	1.000	100	0.10	3
10	2.000	100	0.20	4
10	5.000	100	0.50	5
1000	0.100	100	1.00	6
1000	0.200	100	2.00	7
1000	0.300	100	3.00	8
1000	0.500	100	5.00	9
1000	1.000	100	10.00	10

5.4.3 Quality control standards:

The control standards are prepared from the secondary source stocks. The control concentration is 1.00 µg/mL and is prepared using the same dilutions as used to prepare the 1.00 µg/mL working standards. All dilutions are made using nanopure deionized water. Store the control standards in 50 ml disposable centrifuge tubes in the refrigerator. Control standards are usable for no more than 21 days before they must be prepared again from the stock solutions.

6 Safety

6.1 For general laboratory safety procedures, consult the ARB Laboratory Safety Manual (Chemical Hygiene Plan). Material Safety Data Sheets (MSDS) are available in the laboratory.

7 Procedure

Filter samples are provided by clients. These filters immediately are stored in a freezer until analysis. Samples must be analyzed within 30 days after receipt in the laboratory.

7.1 Filter Extraction and Storage

Prepare a list of samples to be analyzed and the "analyze-by" date. Each batch generally includes no more than 20 samples because IC running time for 20 samples plus standards and QC samples is about a day and the re-calibration of IC is necessary.

7.1.1 Filters are generally contained in individual PetriSlides. Gloves are worn to prevent contamination of the samples from fingers. A pair of tweezers is used to pick up and transfer the filter into the centrifuge tube. The filters are rolled to fit centrifuge tubes and transferred carefully into 15 mL plastic centrifuge tubes without folding. Be sure that the exposed area of the filter is well below the level of the extraction water to be added.

7.1.2 Label four additional centrifuge tubes as 1) extraction water blank, 2) filter blank, 3) anion spike, and 4) cation spike tubes. Place one new same type filter as those used for sampling in the filter blank, and spike tubes. To the anion and cation spike tubes, add 0.632 mL of the 10 µg/mL anion and cation working standard, respectively (final concentration of each ion added is 0.5 µg/mL with 12 mL extraction volume). One extraction water blank, one filter blank, one anion and one cation spike samples are collected for every 20 filter samples (at least one set of water blank, filter blank, anion spike and cation spike per batch of samples).

7.1.3 Verify that the nanopure deionized water dispenser is accurate by dispensing 12.0 ml into a 25 mL glass stopped cylinder previously calibrated by weight. Dispense 12.0 mL fresh nanopure deionized water to all tubes.

7.1.4 Securely replace the lids on each centrifuge tube. Be sure that the exposed area on the filter is completely and continually immersed in the extraction solution.

7.1.5 Place them in a rack inside of the ultrasonic bath that has been filled with laboratory distilled or deionized water above the level of the solution inside the tubes. Sonicate for 60 minutes. The bath temperature should not exceed 27 ºC (add ice to the bath).

7.1.6 After sonicating for 60 minutes, remove the samples from the ultrasonic bath and store in the refrigerator at about 4 ^{o}C overnight to settle particles in extracts. The samples are now ready for IC analysis.

7.2 Ion chromatography analysis

The analyst must be familiar with the operation of the IC system. Detailed operating procedure is in the IC system manual. This procedure just serves as an outline of the IC steps that must be followed.

7.2.1 Fill the appropriate reservoir bottles with fresh solutions (eluent, suppressor water) and cap reservoirs.

7.2.2 Check that the helium regulator is set to 30 psi and the valve is open. Make sure that the pressure for eluent reservoir is about 8 psi.

7.2.3 Check and empty solvent waste reservoir.

7.2.4 Start the analysis programs to run the IC.

7.2.5 Allow the baseline to stabilize. This may take 30 minutes or several hours, depending on what conditions have been changed. If any aspects of the baseline (e.g. noise or drift) have changed significantly (compared with the previous run), investigate before continuing. System is stable when the system pressure and total conductivity matches those of the last valid set of analysis.

7.2.6 Prepare a list for the analytical run that begins with water blank, and a set of calibration standards in order of increasing concentration. Insert water blank between the anion standards and cation standards, followed by water blank, and control standards.

7.2.7 The samples and replicates are analyzed after the water blank is checked, the calibration curve is established and the control standards pass.

7.2.8 To the list, add the samples to be analyzed, including at least 10 percent duplicates. At the end of the samples, extraction water blank, a filter blank and the spike samples are analyzed. The last two analyses of each batch are the control standards.

7.2.9 With disposable pipets, transfer approximately 3 mL each of working standards, controls, blanks, and extracted samples including the duplicate aliquots to the Dionex IC autosampler vials. Cap each vial with a filter cap and place in the autosampler cassettes. Place the cassettes on the autosampler and begin the analysis. The remaining portions of the extracts are stored, refrigerated for archive and then discarded after 6 months.

7.2.10 Start the IC sequence. The IC instrument runs automatically including data processing. A typical IC chromatogram of a filter extract is shown in Figure 1 for anions and Figure 2 for cations.

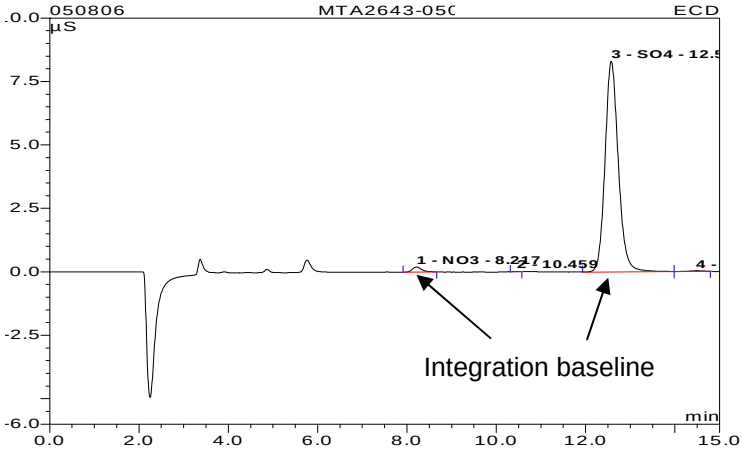


Figure 1 IC chromatogram of anions

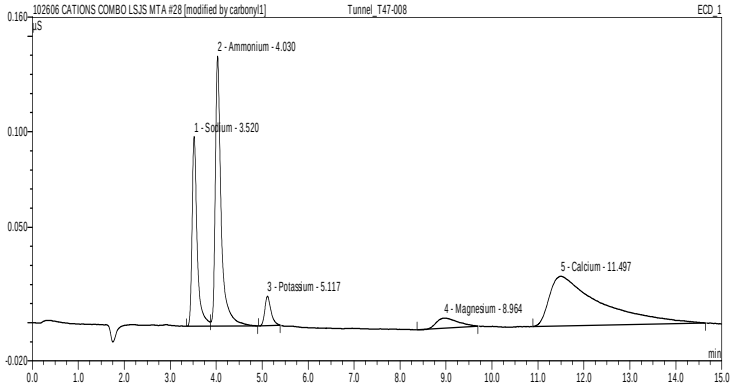


Figure 2 IC chromatogram of cations

8 Quality Control

8.1 The limit of detection (LOD) is defined as the lowest concentration an analyte can be quantified with a certain confidence level. The calculated LOD for the method is determined by analyzing a low concentration standard seven times, as follows:

$$LOD = t_{(n-1,\ 1-\alpha=0.99)} \times \sigma$$

where σ is the standard deviation and is calculated for the seven replicates, and where *t* is the Student's *t* value associated at 99% confidence level. The calculated LOD = 3.143 (n=7) x standard deviation.

8.1.1 The Student's *t* value is dependent upon the degrees of freedom associated with the analysis. The degrees of freedom of the analysis is equal to the number of replicate measurements, n, of the lowest concentration standard minus one. An abbreviated table of values of t associated at 99% confidence level is shown below (Ref. 10.5):

Degrees of Freedom (n-1)	t-value
4	3.747
5	3.365
6	3.143
7	2.998

8.1.2 The LOD is determined at least once per year or after modifications or repair on the instruments which can affect the instrument sensitivity.

8.1.3 The reporting limit is at least 10 times the standard deviation and should include the chemist's judgment on the method and the instrument's variation of performance over time.

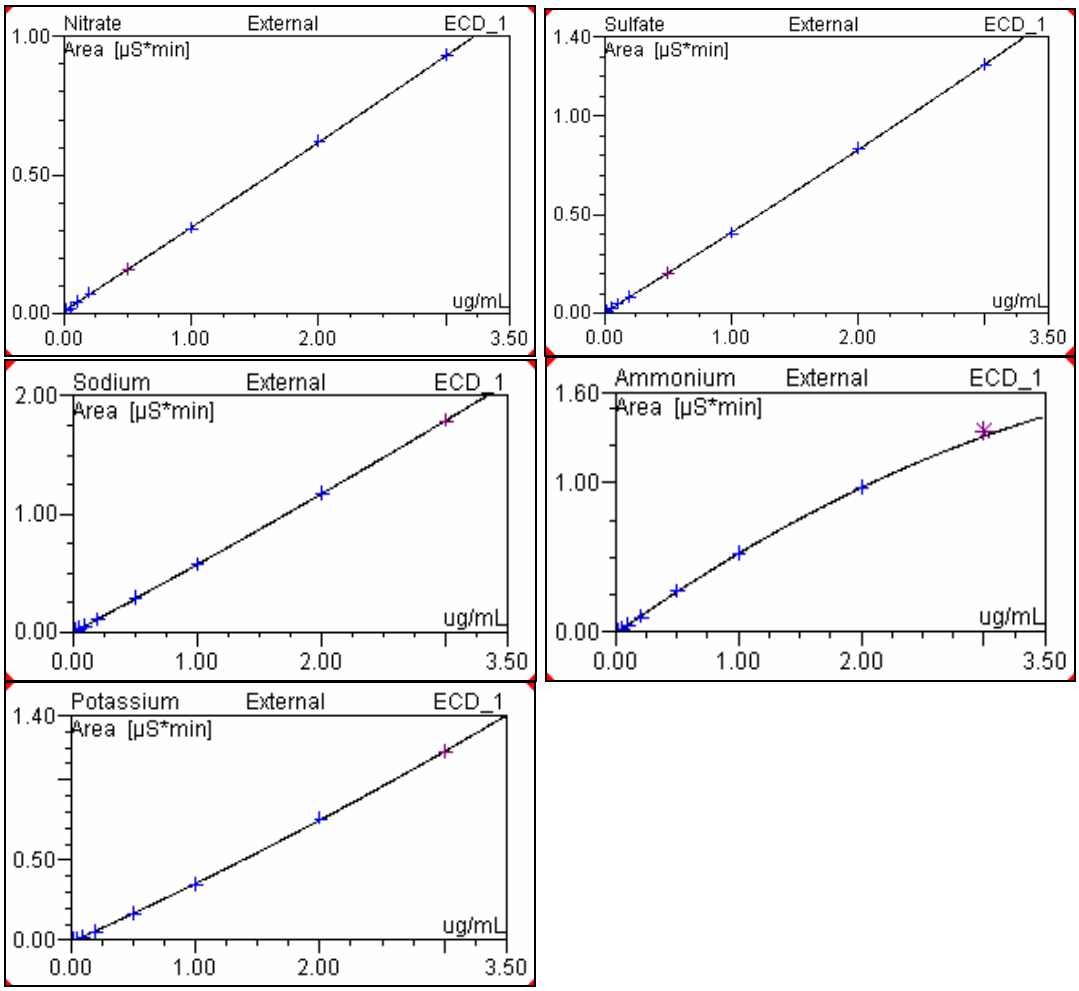
The table below lists the current calculated LOD and reporting limit values.

	Nitrate (µg/mL)	Sulfate (µg/mL)	Sodium (µg/mL)	Ammonium (µg/mL)	Potassium (µg/mL)
Concentration of Lowest Standard Used	0.02	0.02	0.02	0.02	0.02
Standard Deviation	0.0040	0.0035	0.0049	0.0020	0.0015
Calculated LOD	0.013	0.011	0.016	0.006	0.005
Reporting limit	0.040	0.040	0.050	0.040	0.040

8.2 The standards are analyzed for both anion and cation calibration curves. In order for the calibration curve to be acceptable, the correlation coefficient must be greater than or equal to 99% for all ions. If the calibration doesn't meet these requirements, the standards should be re-analyzed or re-prepared, or the instrument should be checked. To get acceptable calibration curve for the ammonium ion, the calibration range used is 0.02 µg/mL - 2.00 µg/mL.

8.3 IC standard calibration curves of target ions.

The following plots are typical examples of the calibration curves.



8.4 Control standards are analyzed after the set of calibration standards are complete and again after each set of samples. The acceptable limits for the control are determined annually using results of control analysis over the previous year. The limits are defined as follows:

Upper Control Limit (UCL)= average value + 3 times the standard deviation
Upper Warning Limit (UWL)= average value + 2 times the standard deviation
Lower Warning Limit (LWL)= average value - 2 times the standard deviation
Lower Control Limit (LCL)= average value - 3 times the standard deviation

8.4.1 Control values outside the upper control limit (UCL) or lower control limit (LCL), are considered to have failed the quality control requirement. The analysis in this situation is referred to

as a "QC failure."

8.4.2 If any of the control values is between control and warning limits (UWL or LWL), it is considered a quality control (QC) "warning". When warnings occur on two consecutive analyses, the second value is considered a QC failure. A QC failure requires that the instrument must be evaluated for problems, and problems corrected if necessary. The control sample must be reanalyzed until the control values are within the control limits, and then sample analysis can proceed.

8.4.3 The initial values for control limits were calculated from results of analysis over the previous months of the program. These values are shown in the table below as an example of the ranges to be expected.

Table of Control Limits:

	Nitrate (µg/mL)	Sulfate (µg/mL)	Sodium (µg/mL)	Ammonium (µg/mL)	Potassium (µg/mL)
Number of control analyzed	20	20	20	20	20
Standard Deviation	0.045	0.026	0.046	0.046	0.043
UCL	1.146	1.106	1.188	1.161	1.159
UWL	1.101	1.080	1.141	1.116	1.116
Average	1.011	1.027	1.049	1.024	1.030
LWL	0.922	0.975	0.956	0.933	0.945
LCL	0.877	0.948	0.910	0.887	0.902

8.5 Nanopure deionized water blanks, extraction water blanks, and filter blanks are analyzed with each set of extracted filters.

8.5.1 If any water blank shows a peak greater than the limit of detection (LOD) in the region of interest, the source of contamination must be investigated and remedied. Blank levels are monitored to assure that contamination from reagents or from sampling processing techniques are not affecting sample results.

8.6 Spikes are run to measure the accuracy of the entire sample handling process. Spikes are prepared from unexposed filters and extraction water with an amount of standard added to bring the final extract concentration to an expected value of 0.50 µg/mL. The spikes are extracted and analyzed with each sample set. The limit for spike recovery efficiency is 100% ± 20% (0.400 – 0.600 µg/mL).

8.7 Ten percent of analyzed extracts are randomly selected and subjected to replicate analysis (at least 2 samples are selected). Replicate analysis consists of a separate aliquot of filter extract solution. The relative percent difference (RPD) in concentrations between the pair of analyses is calculated for each of the target ions.

8.7.1 The RPD is calculated as follows:

$$RPD = \frac{\left|\text{Sample Conc.} - \text{Replicate Conc.}\right|}{\text{Average Conc. of Both Analyses}} \times 100$$

8.7.2 A limit on the allowable RPD is established based on the average concentration of the replicate runs, as shown in the following table:

Average Measurement for Replicate Runs	Allowable RPD (%)
1 to 5 times LOD	Not evaluated
5 to 20 times LOD	<25%
Greater than 20 times LOD	<15%

8.7.3 If the measured RPD of any of the target ions is greater than the allowable limit, the sample is re-analyzed.

9 Calculations

9.1 The software applies the calibration curves to the samples for calculating concentrations. If the concentration of any of the target ions is beyond the concentration range of the calibration curve, these samples are diluted and re-analyzed.

9.2 Transfer the data to an Excel sheet that already contains the sample names, QC sample concentrations, the reporting limit concentrations.

9.3 Calculate the spike standard recoveries and the duplicates' relative differences.

9.4 Confirm that all QC elements are under control. Otherwise, fix problems and re-analyze samples.

9.5 Filter extract concentrations are then used to calculate the total amount of anions and cations on each filter:

$$Mass\ (\mu g) = Concentration\ (\mu g\ /mL)\ x\ Extraction\ Volume\ (mL)$$

10 References

10.1 EPA Method 300.6, Orthophosphate, Nitrate, and Sulfate in Wet Deposition by Chemically Suppressed Ion Chromatography, USEPA, March 1986.

10.2 EPA: Quality Assurance Guidance Document, Final, Quality Assurance Project Plan, PM 2.5 Speciation Trends Network Field Sampling, EPA-4154/R-01-001.

10.3 EPA: Guideline on Speciated Particulate Monitoring August 1998 http://www.epa.gov/ttn/amtic/files/ambient/pm25/spec/drispec.pdf

10.4 ARB SOP MLD 064: Standard Operating Procedure for the Analysis of Anions and Cations in PM 2.5 Speciation Samples by Ion Chromatography.

10.5 Harris, Daniel C., “Quantitative Chemical Analysis”, *W.H. Freeman & Co.*, 4th ed., **1995**